CLAIMS

We claim:

1. A method of generating a population of rAAV particles comprising the step of: incubating a producer cell in a cell culture medium, wherein said producer cell is cultured under suspension conditions, wherein the producer cell comprises: (i) one or more AAV packaging genes, wherein each said AAV packaging gene encodes an AAV replication or encapsidation protein; (ii) a recombinant AAV (rAAV) vector that comprises a heterologous non-AAV polynucleotide flanked by at least one AAV inverted terminal repeat (ITR); and (iii) helper virus function for AAV, and wherein the producer cell is other than a KB cell, whereby greater than about 10^2 particles are produced from the producer cell.

2. A method according to claim 1, further comprising the step of employing tangential flow filtration after virus has been produced.

3. A method of generating a population of recombinant adeno-associated virus (rAAV) particles, comprising the step of:

. incubating an AAV producer cell under conditions that are permissive for replication of AAV, said producer cell comprising (i) one or more AAV packaging genes wherein each said AAV packaging gene encodes an AAV replication or encapsidation protein; (ii) a recombinant AAV (rAAV) vector that comprises a heterologous non-AAV polynucleotide flanked by at least one AAV inverted terminal repeat (ITR); and (iii) a helper virus for AAV, wherein said helper virus is a temperature-sensitive helper virus, wherein the incubating the producer cell line is conducted at a temperature that is permissive for replication of AAV but non-permissive for replication of the temperature-sensitive helper virus, wherein the incubation occurs for at least five days from the time of introduction of the temperature-sensitive adenovirus, whereby AAV virus particles are produced.

4. A method according to claim 3, wherein said temperature sensitive helper virus is adenovirus Ad-ts149.

5 A method according to claim 3, wherein the temperature-sensitive adenovirus is in the form of a plasmid.

6. A method according to claim 3, wherein the temperature-sensitive adenovirus is in the form of a packaged virus particle.

7. A method according to claim 3, wherein rAAV production is increased at least about 5-fold as compared to rAAV production using a wild type adenovirus.

8. A method of isolating a population of rAAV particles, comprising the steps of: (a) chromatographing an AAV producer cell lysate containing rAAV particles on a positively-charged anion exchange resin; and (b) chromatographing an AAV producer cell lysate containing rAAV particles on a negatively-charged cation exchange resin, whereby a purified population of rAAV particles is generated.

9. The method of claim 8, wherein step a is performed before step b.

10. The method of claim 8, wherein step b is performed before step a.

11. The method of claim 10, further comprising step (c) of chromatographing the lysate containing rAAV particles on a negatively-charged cation exchange resin, said step performed after steps (a) and (b).

12. The method of claim 11, wherein heparin sulfate is used for step (c).

13. The method of claim 8, further comprising the step of subjecting the producer cells to tangential flow filtration.

14. The method of claim 8, wherein the lysate is subjected to tangential flow filtration.

15. The method of claim 14, wherein tangential flow filtration is performed prior to chromatography.

16. The method of claim 14, wherein tangential flow filtration is performed after chromatography.

17. The method of claim 8, wherein said anion exchange resin is an N-charged amino or imino resin.

18. The method of claim 17, wherein said anion exchange resin is selected from the group consisting of a POROS 50 PI resin, a diethylaminoethyl (DEAE) resin, a trimethylaminoethyl (TMAE) resin, a quaternary amine resin and a polyethylenimine (PEI) resin.

19. The method of claim 8, wherein said cation exchange resin is a sulfo-, phospho- or carboxy-based cationic resin.

20. The method of claim 19, wherein said cation exchange resin is selected from the group consisting of an HS resin, an SP resin, and a carboxymethyl (CM) resin.

21. The method of claim 8, wherein the producer cell is cultured under suspension conditions.

22. A method of isolating a population of rAAV particles, comprising the steps of: (a) chromatographing AAV producer cell culture supernatant which contains rAAV particles on a positively-charged anion exchange resin; and (b) chromatographing the AAV producer cell culture supernatant containing rAAV particles on a negatively-charged cation exchange resin, whereby a purified population of rAAV particles is generated.

23. The method of claim 22, wherein step a is performed before step b.

24. The method of claim 22, wherein step b is performed before step a.

25. The method of claim 24, further comprising step (c)of chromatographing the lysate containing rAAV particles on a negatively-charged cation exchange resin, said step performed after steps (a) and (b).

26. The method of claim 25 wherein heparin sulfate is used for step (c).

27. The method of claim 22, further comprising the step of subjecting the culture supernatant to tangential flow filtration.

28. The method of claim 27, wherein tangential flow filtration is performed prior to chromatography.

29. The method of claim 27, wherein tangential flow filtration is performed after chromatography.

30. The method of claim 22, wherein said anion exchange resin is an N-charged amino or imino resin.

31. The method of claim 20, wherein said anion exchange resin is selected from the group consisting of a POROS 50 PI resin, a diethylaminoethyl (DEAE) resin, a trimethylaminoethyl (TMAE) resin, a quaternary amine resin and a polyethylenimine (PEI) resin.

32. The method of claim 22, wherein said cation exchange resin is a sulfo-, phospho- or carboxy-based cationic resin.

33. The method of claim 32, wherein said cation exchange resin is selected from the group consisting of an HS resin, an SP resin, and a carboxymethyl (CM) resin.

34. The method of claim 22, wherein the producer cell is cultured under suspension conditions.

35. A method of isolating rAAV particles comprising the steps of (a) chromatographing an AAV producer cell lysate containing rAAV particles on a positively charged anion exchange resin; and (b) subjecting the product of step a to tangential flow filtration to generate a purified population of rAAV.

36. The method of claim 35, wherein step a is performed before step b.

37. The method of claim 35, wherein step b is performed before step a.

38. The method of claim 35, wherein said anion exchange resin is an N-charged amino or imino resin.

39. The method of claim 39, wherein said anion exchange resin is selected from the group consisting of a POROS 50 PI resin, a diethylaminoethyl (DEAE) resin, a trimethylaminoethyl (TMAE) resin, a quaternary amine resin and a polyethylenimine (PEI) resin.

40. The method of claim 35, wherein the producer cell is cultured under suspension conditions.

41. A method of isolating rAAV particles comprising the steps of (a) chromatographing an AAV producer cell culture supernatant which contains rAAV particles on a positively charged anion exchange resin; and (b) subjecting the product of step a to tangential flow filtration to generate a purified population of rAAV.

42. The method of claim 41, wherein step a is performed before step b.

43. The method of claim 41, wherein step b is performed before step a.

44. The method of claim 41, wherein said anion exchange resin is an N-charged amino or imino resin.

45. The method of claim 44, wherein said anion exchange resin is selected from the group consisting of a POROS 50 PI resin, a diethylaminoethyl (DEAE) resin, a trimethylaminoethyl (TMAE) resin, a quaternary amine resin and a polyethylenimine (PEI) resin.

46. The method of claim 41, wherein the producer cell is cultured under suspension conditions.

47. A method of generating a population of rAAV particles comprising culturing a producer cell under a stress condition, said producer cell comprising (i) one or more AAV packaging genes, wherein each said AAV packaging gene encodes an AAV replication or encapsidation protein; (ii) a recombinant AAV (rAAV) vector that comprises a heterologous non-AAV polynucleotide flanked by at least one AAV inverted terminal repeat (ITR); and (iii) helper virus function for AAV, whereby about two-fold or more rAAV particles are produced compared to a producer cell not grown under said stress condition.

48. The method of claim 47, wherein the producer cell is attachment dependent.

49. The method of lcaim 47, wherein the producer cell is grown in suspension.

50. A method of generating a population of recombinant adeno-associated virus (rAAV) particles, comprising the steps of:

a) providing an AAV producer cell that comprises:

(i) one or more AAV packaging genes, wherein each said AAV packaging gene encodes an AAV replication or encapsidation protein;

(ii) a recombinant AAV (rAAV) pro-vector that comprises a heterologous non-AAV polynucleotide flanked by at least one AAV inverted terminal repeat (ITR); and

(iii) a helper virus for AAV;

b) incubating the producer cell provided in step a) under conditions that are permissive for replication of AAV;

c) lysing the producer cell after the incubation of step b) to produce an AAV producer cell lysate;

d) chromatographing the AAV producer cell lysate of step c) on at least one positively-charged anion exchange resin; and

e) purifying the chromatographic fractions containing rAAV particles of step d) by cation exchange chromatography or tangential flow filtration to generate a purified population of rAAV vector particles.

51. A method of generating a population of rAAV particles according to claim 50, wherein said purifying step e) comprises subjecting the fractions to cation exchange chromatography.

52. A method of generating a population of rAAV particles according to claim 50, wherein said purifying step e) comprises subjecting the fractions to tangential flow filtration.

53. A method of generating a population of rAAV particles according to claim 50, wherein said rAAV pro-vector comprises a heterologous non-AAV polynucleotide flanked by two AAV inverted terminal repeats (ITRs).

54. A method of generating a population of rAAV particles according to claim 50, wherein said AAV producer cell comprises at least one AAV packaging gene that is stably integrated into the genome of said AAV producer cell.

55. A method of generating a population of rAAV particles according to claim 50, wherein said providing of the producer cell in step a) comprises introducing the helper virus into the producer cell already introduced with the AAV packaging gene(s) and the rAAV pro-vector.

56. A method of generating a population of rAAV particles according to claim 50, wherein the providing of the producer cell in step a) comprises introducing the rAAV pro-vector and the helper virus simultaneously or sequentially into the producer cell already introduced with the AAV packaging gene(s).

57. A method of generating a population of rAAV particles according to claim 50, wherein the providing of the producer cell in step a) comprises introducing the AAV packaging gene(s) and the rAAV pro-vector simultaneously or sequentially into the host cell already introduced with the helper virus.

58. A method of generating a population of rAAV particles according to claim 50, wherein said AAV producer cell comprises an AAV *rep* gene and an AAV *cap* gene.

59. A method of generating a population of rAAV particles according to claim 50, wherein said AAV *rep* gene and AAV *cap* gene are stably integrated into the genome of said AAV producer cell.

60. A method of generating a population of rAAV particles according to claim 50, wherein the providing of the producer cell in step a) comprises introducing into the producer cell at least one AAV split-packaging gene.

61. A method of generating a population of rAAV particles according to claim 50, wherein said helper virus is an adenovirus.

62. A method of generating a population of rAAV particles according to claim 50, wherein said helper virus is a temperature-sensitive helper virus and said step of incubating the

producer cell is conducted at a temperature that is permissive for replication of AAV but non-permissive for replication of the temperature-sensitive helper virus.

63. A method of generating a population of rAAV particles according to claim 50, wherein said helper virus is a temperature-sensitive adenovirus.

64. A method of generating a population of rAAV particles according to claim 50, wherein said helper virus is adenovirus Ad-ts149.

65. A method of generating a population of rAAV particles according to claim 50, wherein said AAV producer cell lysate is also affinity purified on a resin having a ligand that is specific for one or more surface molecules present on AAV.

66. A method of generating a population of rAAV particles according to claim 65, wherein the affinity purification is conducted after ion-exchange chromatography.

67. A method of generating a population of rAAV particles according to claim 65, wherein said ligand is an antibody that is specific for a surface molecule present on AAV.

68. A method of generating a population of rAAV particles according to claim 50, wherein the AAV producer cells of step b) are concentrated prior to lysis.

69. A method of generating a population of rAAV particles according to claim 68, wherein the AAV producer cells of step b) are concentrated by centrifugation or by tangential flow filtration prior to lysis.

70. A method of generating a population of rAAV particles according to claim 50, wherein said step of lysing the AAV producer cell is conducted by subjecting the cells to a lytic technique selected from the group consisting of microfluidization, sonication, and freeze-thawing.

71. A method of generating a population of rAAV particles according to claim 70, wherein said step of lysing the AAV producer cell is conducted by subjecting the cells to microfluidization.

72. A method of generating a population of rAAV particles according to claim 50, wherein the AAV producer cell lysate of step c) is treated with a nuclease prior to chromatography.

73. A method of generating a population of rAAV particles according to claim 72, wherein said nuclease is Benzonase.

74. A method of generating a population of rAAV particles according to claim 50, wherein the AAV producer cell lysate of step c) is clarified prior to chromatography.

75. A method of generating a population of rAAV particles according to claim 74, wherein the AAV producer cell lysate of step c) is clarified by filtration or centrifugation prior to chromatography.

76. A method of generating a population of rAAV particles according to claim 50, wherein the AAV producer cells are concentrated prior to lysis, resuspended in a buffer comprising saline at an ionic strength at least that of a 50mM NaCl solution, lysed, and then clarified by filtration prior to chromatography.

77. A method of generating a population of rAAV particles according to claim 51, wherein chromatographic fractions containing rAAV particles are concentrated by filtration or centrifugation after elution from the chromatographic resin.

78. A method of generating a population of rAAV particles according to claim 51, wherein chromatographic fractions containing rAAV particles are concentrated by tangential flow filtration

79. A method of generating a population of rAAV particles according to claim 50, wherein said anion exchange resin is an N-charged amino or imino resin.

80. A method of generating a population of rAAV particles according to claim 50, wherein said anion exchange resin is selected from the group consisting of a POROS 50 PI resin, a diethylaminoethyl (DEAE) resin, a trimethylaminoethyl (TMAE) resin, a quaternary amine resin and a polyethylenimine (PEI) resin.

81. A method of generating a population of rAAV particles according to claim 51, wherein said cation exchange resin is a sulfo-, phospho- or carboxy-based cationic resin.

82. A method of generating a population of rAAV particles according to claim 51, wherein said cation exchange resin is selected from the group consisting of an HS resin, an SP resin, and a carboxymethyl (CM) resin.

83. A method of generating a population of rAAV particles according to claim 50, wherein the producer cell of step a) is an attachment-dependent mammalian cell line.

84. A method of generating a population of rAAV particles according to claim 50, wherein said step b) of incubating the producer cell provided in step a) is conducted in a vessel selected from the group consisting of a tissue culture flask, a roller bottle, a spinner flask, a tank reactor, a fermentor, and a bioreactor.

85. A method of generating a population of rAAV particles according to claim 50, wherein said step b) of incubating the producer cell provided in step a) is conducted using a microcarrier.

86. A method of generating a population of rAAV particles according to claim 50, wherein said vessel is a hollow-fiber, packed-bed or fluidized-bed bioreactor.

87. A method of generating a population of rAAV particles according to claim 50, wherein the producer cell of step a) is a suspension-adapted mammalian cell line.

88. A method of generating a population of rAAV particles according to claim 50, wherein said step b) of incubating the producer cell provided in step a) is conducted in a vessel selected from the group consisting of a spinner flask, a tank reactor and an air lift fermentor.

89. A method of generating a population of rAAV particles according to claim 50, wherein said step b) of incubating the producer cell provided in step a) is performed in rAAV medium essentially as shown in Table 2.

90. A method of generating a population of rAAV particles according to claim 50, wherein the producer cells are 293 N3s cells or HeLa S3 cells.

91. A method of generating a population of rAAV particles according to claim 50, wherein step b) is conducted for at least 5 days.

92. A method of generating a population of rAAV particles according to claim 50, wherein step b) of incubating the producer cell is conducted in a multi-liter bioreactor and wherein at least about 10^9 replicative units of rAAV per liter of bioreactor volume are isolated after step e).

93. A method of generating a population of recombinant adeno-associated virus (rAAV) particles, comprising the steps of:

a) providing an AAV producer cell that comprises:

(i) one or more AAV packaging genes, wherein each said AAV packaging gene encodes an AAV replication or encapsidation protein;

(ii) a recombinant AAV (rAAV) pro-vector that comprises a heterologous non-AAV polynucleotide flanked by at least one AAV inverted terminal repeat (ITR); and

(iii) a helper virus for AAV or a polynucleotide sequence of said helper virus that encodes at least one helper virus function;

b) subjecting the producer cell provided in step a) to a sub-lethal stress; and

c) incubating the stressed producer cell of step b) under conditions that are permissive for replication of AAV.

94. A method of generating a population of rAAV particles according to claim 93, wherein said sub-lethal stress is selected from the group consisting of a nutritional stress, an osmotic stress, a pH stress, a temperature stress, an aerobic stress, a mechanical stress, a radiational stress and a toxic stress.

95. A method of generating a population of rAAV particles according to claim 93, wherein said sub-lethal stress is a nutritional stress.

96. A method of generating a population of rAAV particles according to claim 93, wherein said sub-lethal stress is an osmotic stress.

97. A method of generating a population of rAAV particles according to claim 93, wherein said sub-lethal stress is a pH stress.

98. A method of generating a population of rAAV particles according to claim 97, wherein said pH stress comprises raising the pH to above pH 7.2.

99. A method of generating a population of rAAV particles according to claim 97, wherein said pH stress comprises elevating the pH to at least 7.4, and wherein the majority of the AAV particles produced are released into the supernatant.

100. A method of generating a population of rAAV particles according to claim 97, wherein said pH stress comprises elevating the pH to about 8.0.

101. A method of generating a population of rAAV particles according to claim 93, wherein said sub-lethal stress is a temperature stress.

102. A method of generating a population of rAAV particles according to claim 93, wherein said sub-lethal stress is an aerobic stress.

103. A method of generating a population of rAAV particles according to claim 93, wherein said sub-lethal stress is a mechanical stress.

104. A method of generating a population of rAAV particles according to claim 93, wherein said sub-lethal stress is a radiational stress.

105. A method of generating a population of rAAV particles according to claim 93, wherein said sub-lethal stress is a toxic stress.

106. A method of generating a population of rAAV particles according to claim 95, wherein said nutritional stress is imposed by culturing the producer cells in a medium that is deficient in one or more amino acids.

107. A method of generating a population of rAAV particles according to claim 95, wherein said nutritional stress is imposed by culturing the producer cells in a medium that is deficient in aspartic acid.

108. A method of generating a population of rAAV particles according to claim 95, wherein said nutritional stress is imposed by culturing the producer cells in a medium that is deficient in glutamic acid.

109. A method of generating a population of rAAV particles according to claim 108, wherein the deficient medium contains less than 10 μmol/L of aspartic acid.

110. A method of generating a population of rAAV particles according to claim 95, wherein the deficient medium contains less than 2 μmol/L of glutamic acid.

111. A method of generating a population of rAAV particles according to claim 95, wherein said nutritional stress is imposed by culturing the producer cells in a medium that is deficient in serum.

112. A method of generating a population of rAAV particles according to claim 95, wherein the cells are subjected to said nutritional stress by introducing the cells into a nutritionally deficient medium.

113. A method of generating a population of rAAV particles according to claim 95, wherein the cells are subjected to said nutritional stress by culturing the cells in a medium until the medium becomes nutritionally deficient.

114. A method of generating a population of rAAV particles according to claim 93, wherein said purified population of rAAV vector particles is substantially free of replication-competent AAV and of helper virus and cellular proteins.

115. A method of generating a population of rAAV particles according to claim 93, in which elution from the chromatographic resin is conducted by increasing the salt concentration and chromatographic eluants comprising rAAV particles are subsequently treated to reduce the effective salt concentration by dilution, dialysis, diafiltration or concentration.

116. A method of generating a population of rAAV particles according claim 93, including the step of subjecting a fraction comprising AAV particles to heparin sulfate chromatography.

117. A method of generating a population of recombinant adeno-associated virus (rAAV) particles, comprising the steps of:

a) providing an AAV producer cell that comprises:

(i) one or more AAV packaging genes, wherein each said AAV packaging gene encodes an AAV replication or encapsidation protein;

(ii) a recombinant AAV (rAAV) pro-vector that comprises a heterologous non-AAV polynucleotide flanked by at least one AAV inverted terminal repeat (ITR); and

(iii) a helper virus for AAV;

b) incubating the producer cell provided in step a) under conditions that are permissive for replication of AAV and which comprise inducing a sub-lethal stress in the AAV producer cell;

c) lysing the producer cell after the incubation of step b) to produce an AAV producer cell lysate; and

d) purifying the AAV producer cell lysate to generate a population of recombinant adeno-associated virus (rAAV) particles.

118. A method of generating a population of rAAV particles according to claim 117, wherein said purifying step d) comprises chromatographing the AAV producer cell lysate of step c) on at least one positively-charged anion exchange resin followed by purifying on either a cation exchange resin or by tangential flow filtration to generate a purified population of rAAV vector particles.

119. The method of claim 118, wherein said purifying step d) comprises chromatographing the AAV producer cell lysate of step c) on at least one negatively -charged cation exchange resin followed by purifying on an anion exchange resin.

120. A method of generating a population of rAAV particles according to claim 117, wherein said purifying step d) comprises chromatographing the AAV producer cell lysate of step

c) on a positively-charged anion exchange resin followed by tangential flow filtration to generate a purified population of rAAV vector particles.

121. A method of generating a population of rAAV particles according to claim 117, wherein said rAAV pro-vector comprises a heterologous non-AAV polynucleotide flanked by two AAV inverted terminal repeats (ITRs).

122. A method of generating a population of rAAV particles according to claim 117, wherein said AAV producer cell comprises at least one AAV packaging gene that is stably integrated into the genome of said AAV producer cell.

123. A method of generating a population of rAAV particles according to claim 117, wherein said AAV producer cell comprises an AAV *rep* gene and an AAV *cap* gene.

124. A method of generating a population of rAAV particles according to claim 117, wherein said helper virus is adenovirus.

125. A method of generating a population of virus particles, comprising the step of:

a) incubating a producer cell in a cell culture medium under conditions comprising a condition that promotes release of virus particles, whereby virus particles are released from the producer cell into the culture medium.

126. The method of claim 125, wherein the virus is recombinant adeno-associated virus (rAAV), and wherein the producer cell comprises:

(i) one or more AAV packaging genes, wherein each said AAV packaging gene encodes an AAV replication or encapsidation protein;

(ii) a recombinant AAV (rAAV) vector that comprises a heterologous non-AAV polynucleotide flanked by at least one AAV inverted terminal repeat (ITR); and

(iii) helper virus function for AAV.

127. The method of claim 126, wherein the condition that promotes release of virus particles is pH.

128. The method of claim 127, wherein the pH is about 7.4 to about 8.0.

129. The method of claim 128, wherein the pH is about 8.0.

130. The method of claim 126, wherein the condition that promotes virus release is osmolality.

131. The method of claim 130, wherein the osmolality is about 300 mOsm.

132. The method of claim 131, wherein the pH is about 8.00.

133. The method of claim 132, wherein the pH is maintained at about 8.00.

134. The method of claim 133, wherein the pH is adjusted by using a sodium salt.

135. The method of claim 131, wherein the osmolality is adjusted using an ionic salt.

136. The method of claim 135, wherein the ionic salt is NaCl.

137. The method of claim 130, wherein the initial osmolality of the cell culture is about 300 mOsm.

138. The method of claim 137, wherein the pH is about 8.00.

139. The method of claim 137, wherein the osmolality is adjusted using an ionic salt.

140. The method of claim 139, wherein the ionic salt is NaCl.

141. The method of claim 126, wherein the condition that promotes virus release is temperature.

142. The method of claim 141, wherein the temperature is about 37°C to about 40°C.

143. The method of claim 142, wherein the temperature is about 39°C.

144. The method of claim 133, wherein the temperature is about 39°C.

145. The method of claim 144, wherein the osmolality is about 300 to about 350 mOsm.

146. The method of claim 137, wherein the temperature is about 39°C.

147. The method of claim 126, wherein the condition that promotes release of virus particles is conductivity.

148. The method of claim 147, wherein the conductivity is at least about 10 mS.

149. The method of claim 147, wherein the conductivity is about 10 mS.

150. The method of claim 147, wherein the conductivity is about 15 mS.

151. The method of claim 147, wherein the conductivity is adjusted using a sodium salt.

152. The method of 151, wherein the sodium salt is NaCl.

153. The method of claim 126, wherein the condition that promotes release of virus particles is an agent or condition that permeabilizes the producer cell.

154. The method of claim 133, wherein producer cells are cultured for about 48 to about 96 hours after introduction of helper virus function.

155. The method of claim 143, wherein producer cells are cultured for about 48 to about 96 hours after introduction of helper virus function.

156. The method of claim 126, wherein helper virus function is provided by helper virus.

157. A method of generating a population of rAAV particles according to claim 156, wherein said helper virus is an adenovirus.

158. The method of claim 126, further comprising the step of (b) harvesting the viral particles from the cell culture medium, thereby obtaining a population of rAAV particles.

159. The method of claim 158, further comprising the steps of:

c) chromatographing the AAV producer cell culture medium on a positively-charged anion exchange resin; and

d) purifying the chromatographic fractions containing rAAV particles of step c) by cation exchange chromatography or tangential flow filtration to generate a purified population of rAAV vector particles.

160. The method of claim 159, wherein step (d) is cation exchange chromatography.

161. The method of claim 158, further comprising the steps of

c) chromatographing the AAV producer cell culture medium on a negatively-charged cation exchange resin;

d) purifying the chromatographic fractions containing rAAV particles of step c) by anion exchange chromatography; and

e) purifying the chromatographic fractions containing rAAV particles of step d) by cation exchange chromatography to generate a purified population of rAAV vector particles.

162. The method of claim 161, wherein the chromatography of step e) is performed using heparin sulfate.

163. A method of generating a population of rAAV particles according to claim 126, wherein said rAAV vector comprises a heterologous non-AAV polynucleotide flanked by two AAV inverted terminal repeats (ITRs).

164. A method of generating a population of rAAV particles according to claim 126, wherein said AAV producer cell comprises at least one AAV packaging gene that is stably integrated into the genome of said AAV producer cell.

165. A method of generating a population of rAAV particles according to claim 126, wherein said AAV producer cell comprises an AAV *rep* gene and an AAV *cap* gene.

166. A method of generating a population of rAAV particles according to claim 165, wherein said AAV *rep* gene and AAV *cap* gene are stably integrated into the genome of said AAV producer cell.

167. A method of generating a population of rAAV particles according to claim 126, wherein the producer cell is an attachment-dependent mammalian cell line.

168. A method of generating a population of rAAV particles according to claim 126, wherein the producer cell is a suspension-adapted mammalian cell line.

169. A high-throughput assay for determining the infectious titer of a preparation containing a virus that can replicate in a mammalian cell, comprising the steps of:

a) providing an array of culture wells each comprising an aliquot of mammalian cells and an aliquot of the virus preparation to be titered;

b) incubating the cells and virus of step a) to allow replication of said virus;

c) lysing said cells to produce a multiplicity of lysates containing viral polynucleotides;

d) transferring the multiplicity of lysates from step c) to a membrane that binds nucleic acids to produce a membrane-bound array of nucleic acids;

e) hybridizing the membrane-bound array of nucleic acids of step d) with a viral-specific probe and then determining the relative amount of viral nucleic acid replicated in each of said culture wells.

170. A high-throughput assay for determining the infectious titer of a virus preparation according to claim 169, wherein said virus is adenovirus or AAV.

171. A high-throughput assay for determining the infectious titer of a virus preparation according to claim 169, wherein said virus is AAV.

172. A high-throughput assay for determining the infectious titer of a virus preparation according to claim 169, wherein said array of culture wells is in the form of a microtiter vessel.

173. A high-throughput assay for determining the infectious titer of a virus preparation according to claim 169, wherein said aliquots of virus preparation are serially diluted aliquots.

174. A high-throughput method of screening for agents that affect infectivity and/or replication of a virus in a mammalian cell, comprising the steps of:

a) providing an array of culture wells each comprising an aliquot of mammalian cells, an aliquot of the virus and an optionally an aliquot of the agent;

b) incubating the cells, virus, and optional agent of step b) to allow replication of said virus;

c) lysing said cells to produce a multiplicity of lysates containing viral polynucleotides;

d) transferring the multiplicity of lysates from step c) to a membrane that binds nucleic acids to produce a membrane-bound array of nucleic acids;

e) hybridizing the membrane-bound array of nucleic acids of step d) with a viral-specific probe and then determining the relative amount of viral nucleic acid replicated in each of said culture wells.

175. A high-throughput method of screening for agents that affect replication of a virus according to claim 174, wherein said virus is adenovirus or AAV.

176. A high-throughput method of screening for agents that affect replication of a virus according to claim 174, wherein said virus is AAV.

177. A high-throughput method of screening for agents that effect replication of a virus according to claim 174, wherein said array of culture wells is in the form of a microtiter vessel.